Certificate of Analysis

Methylated Human Control

Part# 9PIN123

Revised 9/16

Cat.#	Size
N1231	5µg

Description: Methylated Human Control[a] DNA was purified from a human male source. CpG sites in the DNA were enzymatically methylated by M.SssI methyltransferase to provide a high percentage of methylated CpG motifs.

Storage Conditions: Store at 2–10°C.

Usage Note: Methylated Human Control DNA can be bisulfite-converted with MethylEdge™ Bisulfite Conversion System (Cat.# N1301) in parallel with experimental samples to assess conversion efficiency.

Expiration Date: See product label for expiration date.

Concentration: See the product label for lot-specific information.

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Quality Control Assays

This lot passes the following quality control specifications:

Percent Methylation: ≥ 95% methylation of CpG sites as determined by DNA sequencing.

Signed by:

R. Wheeler, Quality Assurance

[a]Use of Methylation Specific PCR (MSP) is protected by U.S. Pat. Nos. 5,786,146; 6,017,704; 6,200,756 and 6,265,171. No license under these patents to use the MSP process is conveyed expressly or by implication to the purchaser by the purchase of this product.

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Usage Information

1. Purpose

When studying DNA methylation using bisulfite conversion, it is essential that control reactions are run at every step in the process because the presence of a cytosine following bisulfite-conversion indicates methylation. Control DNA should be bisulfite-converted in parallel with experimental samples to ensure that >99% of cytosines are converted and >99% of methylated CpGs are protected. Impurities carried over during purification of source DNA or the presence of secondary structure can affect the efficiency of conversion. Bisulfite-converted control DNA also should be run in parallel with experimental samples in downstream analysis to prevent false-positive identification of methylated cytosines.

2. Bisulfite Conversion

Methylated Human Control DNA[a] (Cat.# N1231) can be bisulfite-converted using systems such as the MethylEdge™ Bisulfite Conversion System. Although 50pg–1µg can be converted in a single reaction, we recommend using 1–2µl of Methylated Human Control DNA. The concentration following bisulfite conversion can be estimated using a UV absorbance scan and viewing the spectra from 220–350nm using absorbance at 260nm. **Note:** If you are using a NanoDrop® Spectrophotometer, set the Sample Type to "RNA-40" because the converted sample contains uracil and is largely single-stranded.

3. Amplification of Bisulfite-Converted DNA

We recommend using either GoTaq® Hot Start Green Master Mix or GoTaq® qPCR Master Mix. Thaw the Master Mix and gently vortex. Both of these master mixes are Hot Start, so the reaction mixes can be set up at room temperature. We recommend amplifying 1-2µl of Converted Methylated Human Control DNA[a] (Cat# N1221) per reaction in parallel with experimental samples.

Suggested Rection Mix

Component	Volume	Final Concentration
2X PCR Master Mix	12.5µl	1X
upstream primer	Xµl	0.2–0.9µM
downstream primer	Xµl	0.2–0.9µM
DNA template	1–2µl	20–50ng
Nuclease-Free Water	Xµl to a final volume of 25µl	

Note: Although typically not necessary, optimizing the magnesium concentration might improve the yield for some targets. When supplementing with magnesium, adjust the Nuclease-Free Water volume to maintain a final volume of 25µl.

Table 1. Cycling Conditions for Endpoint PCR.

Step	Temperature	Time	Number of Cycles
Enzyme Activation	95°C	2 minutes	1
Denaturation	95°C	15 seconds	40
Annealing	Variable	30–60 seconds	
Extension	72°C	60 seconds	
Final Extension	72°C	5 minutes	1

Table 2. Cycling Conditions for Real-Time PCR.

Step	Temperature	Time	Number of Cycles
Enzyme Activation	95°C	2 minutes	1
Denaturation	95°C	15 seconds	40
Annealing	Variable	30–60 seconds	
Dissociation*	65–95°C	variable	1

*Optional

4. Recommendations for PCR Primer Designs

General Considerations

Primer design is key to analyzing bisulfite-converted DNA using PCR-based methods. Primers must be carefully designed based on the converted sequence to avoid PCR bias. Keep in mind that following conversion, DNA strands are no longer complementary and, because the DNA sequence is now reduced to essentially three bases (A, U, G), there is higher probability for non-specific interaction. Unconverted DNA should be run in parallel with bisulfite-converted DNA to ensure the primers are specific to the bisulfite-converted sequence. Several tools are available online to assist in developing primers specific to bisulfite-converted DNA, such as MethPrimer (**www.urogene.org/methprimer/index1.html**).

Primers for experimental DNA samples of poor quality (e.g., DNA isolated from FFPE tissue) should be designed to yield amplicons smaller than 200bp.

Real-Time PCR Considerations

Because bisulfite conversion results in highly fragmented DNA, smaller amplicons will yield better results. Amplicons for real-time PCR should be 75–200bp. If larger amplicons are required, be sure to optimize reaction conditions using control DNA to verify efficiency.

End-Point PCR Considerations

The MethylEdge™ Bisulfite Conversion System (Cat.# N1301) yields bisulfite-converted DNA with significantly less fragmentation than other bisulfite conversion kits . When using this system, amplicons for end-point PCR can be designed up to 500bp when high-quality, purified genomic DNA is used. Amplicons larger than 700bp have been successfully amplified with highly optimized primer models. Amplification of longer sequences may require more template DNA and/or higher primer concentration.

5. Related Products

Product	Size	Cat.#
MethylEdge™ Bisulfite Conversion System	50 reactions	N1301
Converted Methylated Human Control	1µg	N1221
ReliaPrep™ FFPE gDNA Miniprep System	10 reactions	A2351
	100 reactions	A2352
ReliaPrep™ Blood gDNA Miniprep System	100 preps	A5081
	250 preps	A5082
ReliaPrep ™ gDNA Tissue Miniprep System	100 preps	A2051
	250 preps	A2052
GoTaq® Hot Start Green Master Mix	10 reactions	M5121
	100 reactions	M5122
	1,000 reactions	M5123
GoTaq® qPCR Master Mix	200 reactions	A6001
	1,000 reactions	A6002
QuantiFluor® dsDNA System	1ml	E2670
QuantiFluor® ssDNA System	1ml	E3190

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